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Studies on Croatian *Basidiomycota* 1: *Gerhardtia piperata* (*Agaricales*)

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Abstract — *Gerhardtia piperata* is recorded as new to Croatian mycobiota. Black and white photographs of fresh basidiomata and microscopic characters accompany a complete description. The genus *Gerhardtia* is reported from Croatia for the first time.

Key words — *Lyophyllaceae*, biodiversity, biogeography, taxonomy

Introduction

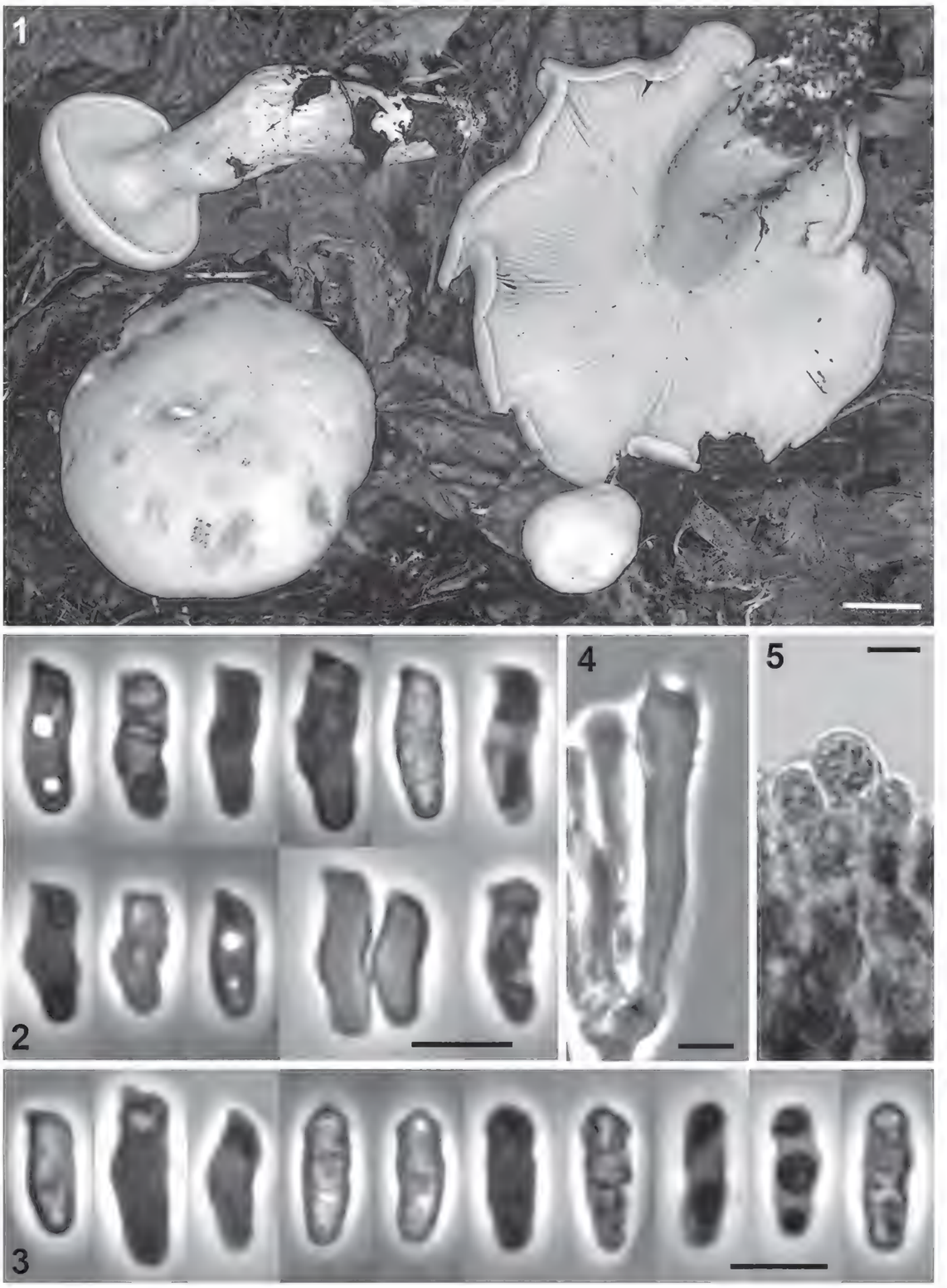
The Croatian Mycological Society, in cooperation with Ruđer Bošković Institute, has carried out the "Biodiversity of Croatian Fungi" project for ten years now (former name: Recording and Mapping of Croatian Fungi). The aim of the project is to determine which fungal species inhabit Croatia, their distribution, and their relation with the environment. As the first step of the project, surveys were made of all species recorded for Croatia representing the *Agaricales* (in the classical sense) up to 2000 (Mešić & Tkalčec 2002, 2003; Tkalčec & Mešić 2002, 2003a,b) and the gastroid *Basidiomycota* up to 2004 (Tkalčec et al. 2005b). The second phase involves intensive field research of Croatian mycobiota. The many valuable results achieved thus far include publication of three new species and a new variety (Hausknecht et al. 2007, Mešić & Tkalčec 2008, Tkalčec & Mešić 2008, Tkalčec et al. 2005a, 2009). Future significant results will be published in a series of scientific papers of which this is the first. This description of the rare species, *Gerhardtia piperata* (*Lyophyllaceae, Agaricales*), is the first record of a member of this genus in Croatia.

Gerhardtia piperata was described by A.H. Smith under the name *Clitocybe piperata* (Smith 1944). Harmaja (1974) transferred the species to *Rhodocybe* due to its undulate and cyanophilous spore wall, under the new name *Rhodocybe smithii*, because of the existence of the earlier similar name *R. piperita* (G. Stev.)

E. Horak. After that, Harmaja (1979) noticed siderophilous granulation in basidia of the holotype specimen and transferred the species to *Lyophyllum*, although *G. piperata* lacks clamp connections (unlike all other members of the genus). Gerhardt (1982) described a species of *Lyophyllum* without clamp connections, *L. incarnatobrunneum* Ew. Gerhardt, and placed it in the new subgenus *Lyophyllopsis* Ew. Gerhardt. Bon (1994) raised subgenus *Lyophyllopsis* to the generic level under the new name *Gerhardtia* Bon with two species included, *G. piperata* and *G. incarnatobrunnea* (Ew. Gerhardt) Bon (according to several authors this species is conspecific with *G. borealis* (Fr.) Contu & A. Ortega which name has priority, e.g. Contu & Ortega 2001, Kalamees 2008). Contu & Consiglio (2005) included four more species with uneven spore wall and no clamp connections in the genus, *G. highlandensis* (Hesler & A.H. Sm.) Cons. & Contu, *G. leucopaxilloides* (H.E. Bigelow & A.H. Sm.) Cons. & Contu, *G. marasmioides* (Singer) Cons. & Contu, *G. suburens* (Clémençon) Cons. & Contu. Here we accept *Gerhardtia,* rather than submerging it in *Lyophyllum.* Phylogenetic analyses of the *Lyophyllaceae* (Hofstetter et al. 2002) based on nuclear and mitochondrial rDNA sequences showed that the generic concept of *Lyophyllum* based only on morphological characters (e.g. Singer 1986) is artificial and should be rearranged. Unfortunately, no species of *Gerhardtia* were included in the study.

Materials and methods

Our description of *Gerhardtia piperata* is based on Croatian records, six collections (consisting of 21 basidiomata) and two records without collected material. The photograph of basidiomata was taken in the field. The description of macroscopic characters is based on observations of fresh material. Color codes (given in brackets) are given according to Kornerup & Wanscher (1981). Specimens were preserved by drying. Microscopic features were observed with a light microscope (bright field and phase contrast) at magnifications up to 1500× and photographed with a digital camera. The morphological description and photographs of microscopic characters were made from rehydrated dried specimens mounted in 2.5% potassium hydroxide solution (KOH), except for spore analysis which was made in 10% ammonia solution (NH_4OH). Amyloidity and dextrinoidity were tested in Melzer's reagent, spore metachromasy in cresyl blue, spore cyanophily in cotton (aniline) blue, and basidium siderophilous granulation n acetocarmine following procedures detailed in Erb & Matheis (1983). Basidiospore measurements were calculated from calibrated digital photographs of spores taken from spore prints of two collections (CNF 1/2633, CNF 1/2677) in which 50 randomly selected spores per print (100 spores in total) were measured without the apiculus. In addition, 50 randomly selected spores were measured from the lamellae of one holotype basidioma. Spore measurements (length, width) are given as: (min.) stat. min.–*av.*–stat. max. (max.), where "min." = minimum (lowest measured value), "stat. min." = statistical minimum (arithmetic average minus two times standard deviation), "*av.*" = arithmetic average, "stat. max." = statistical maximum (arithmetic average plus



Figs. 1–5. *Gerhardtia piperata.* 1. Basidiomata in situ. 2–3. Spores (phase contrast). 4. Basidium (phase contrast). 5. Young basidia with siderophilous granulation. Bars: 1 = 20 mm, 2-5 = 5 µm.

two times standard deviation), "max." = maximum (highest measured value). The range of arithmetic averages (av.) of spore measurements of each particular collection is also given. Standard deviation (SD) of spore length and width is given as: min.–total–max.,

where "min." = collection with lower SD value, "total" = SD value of all 100 measured spores, and "max." = collection with higher SD value. The length/width ratio of spores is given as the "Q" value (min.–av.–max.), and the range of arithmetic averages of "Q" value (Q av.) of each particular collection is also given. Croatian collections with accompanied data are deposited at the Croatian National Fungarium in Zagreb (CNF).

Taxonomic description

Gerhardtia piperata (A.H. Sm.) Bon, Doc. Mycol. 24(93): 67, 1994. FIGS 1–11

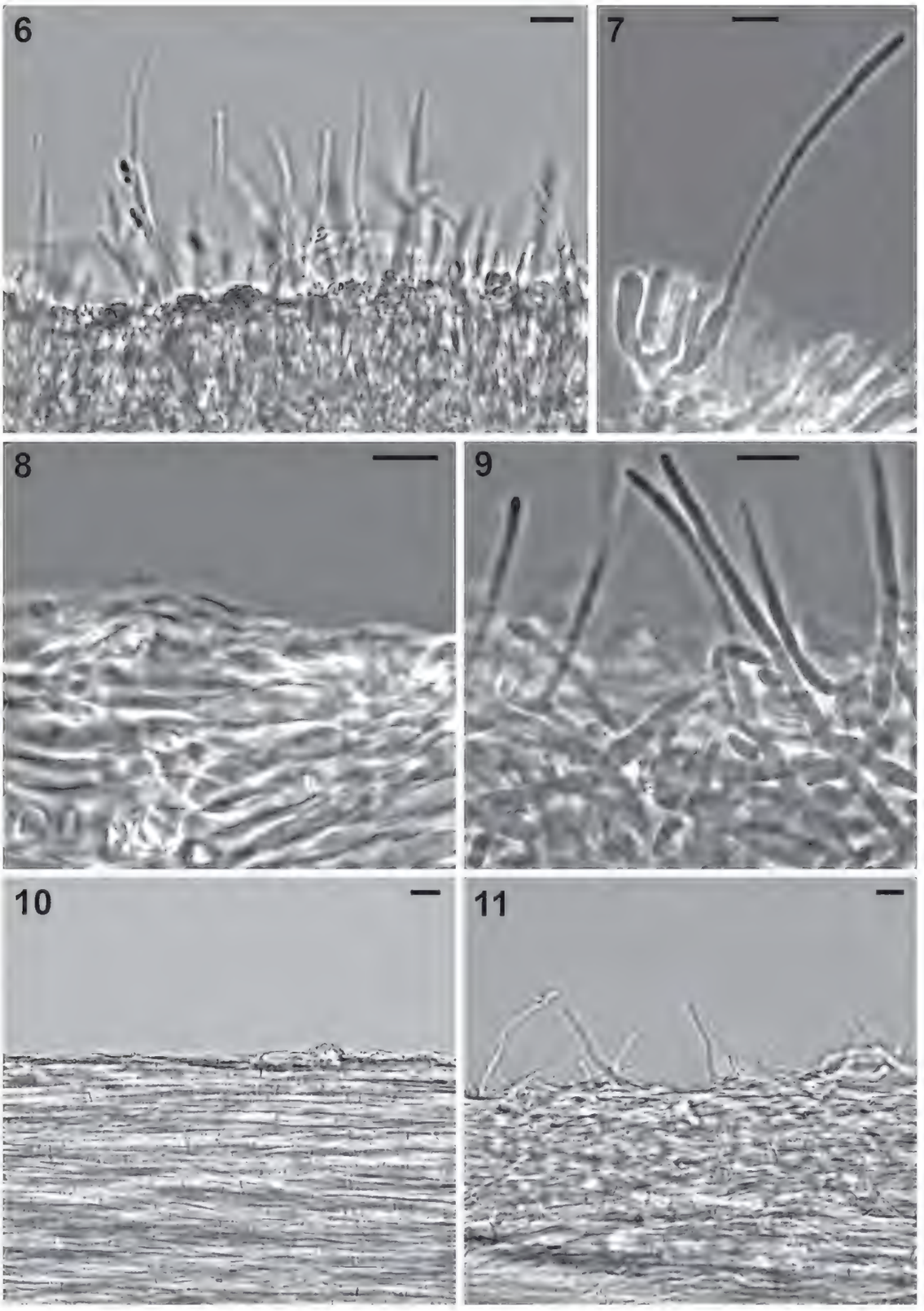
≡ *Clitocybe piperata* A.H. Sm., Bull. Torrey Bot. Club 71: 403, 1944. (basionym)

≡ *Rhodocybe smithii* Harmaja, Karstenia 14: 121, 1974.

≡ *Lyophyllum piperatum* (A.H. Sm.) Harmaja, Karstenia 19(1): 24, 1979.

PILEUS 56–120 mm broad, convex at first, expanding to plano-convex or applanate, sometimes wavy or somewhat lobed, margin involute to inflexed for a long time, not or hardly hygrophanous, not translucently striate, cream (3A2, 4A2) to dirty yellowish (3A3, 4A3), sometimes light brown when old, occasionally with darker watery spots, surface dry, dull, glabrous. LAMELLAE adnate to decurrent, crowded, narrow, often transvenose, sometimes furcate, cream, pale yellowish brown or brownish grey, with entire to undulate, concolorous edge. STIPE 47–103 × 13–30 mm, subcylindrical or tapering towards base, sometimes thickened in the middle, whitish to pale yellowish brown, surface glabrous, fibrillose to finely tomentose, sometimes with scattered minute scales, dry, solid. CONTEXT whitish to cream. SMELL fungoid with strong to weak flowery, perfume or fruity component when cut, often disagreeable. TASTE acrid after few seconds. SPORE PRINT yellowish cream (2A2).

SPORES (5.5–)5.3–*6.6*–7.8(–8.5) × (2.1–)2.0–*2.4*–2.8(–3.1) µm, av. 6.4–6.7 × 2.4–2.4 µm; SD = 0.51–*0.62*–0.71 × 0.20–*0.20*–0.20, Q = 2.26–*2.72*–3.11, Q av. = 2.67–2.77, subcylindrical to subfusiform in side view, often somewhat allantoid or flexuose, subcylindrical to cuneiform in frontal view, surface mostly ± undate to nodose, without germ pore, hyaline, thin-walled, non-amyloid, non-dextrinoid, slightly to distinctly cyanophilous, some spores metachromatic (blue colored with light purple wall). BASIDIA (18–)25–34 × 5–7 µm, narrowly clavate, 4-spored, thin-walled, clampless, with siderophilous granulation. LAMELLAR EDGE fertile to heterogeneous. CHEILOCYSTIDIA absent, rare or locally abundant, 25–100 µm long, 1.8–3(–4) µm broad in upper part, 3-7 µm broad in basal part, (sub)cylindrical, filiform or narrowly lageniform (mostly with long, cylindrical neck), sometimes distorted under the angle or with subcapitate apex, at times with one or two septa, thin-walled, hyaline. PLEUROCYSTIDIA absent. HYMENOPHORAL TRAMA regular to subregular, composed of hyaline, thin-walled to moderately thick-walled (walls up to 0.8 µm thick), subcylindrical to inflated elements, 15–140 × 2–13(–18) µm. PILEIPELLIS a cutis with rare to abundant erect hyphae, in places dense erect hyphae forming a trichoderm,



Figs. 6–11. *Gerhardtia piperata.* 6. Lamellar edge. 7. Cheilocystidium (phase contrast). 8. Pileipellis - cutis (phase contrast). 9. Pileipellis - trichoderm (phase contrast). 10. Stipitipellis - cutis. 11. Stipitipellis - cutis with erect hyphae. Bars = 10 μm.

repent hyphae hyaline, thin-walled (walls up to 0.5 µm thick), cylindrical to inflated, 1.5–7(–12) µm broad, sometimes with lateral projections, erect hyphae (sub)cylindrical to filiform, hyaline, thin-walled, up to 140 µm long, 1.5–3(–4) µm broad. Pileal trama composed of hyaline, thin-walled to moderately thick-walled (walls up to 0.8 µm thick), subcylindrical to inflated, 2–20 µm broad elements. Stipitipellis similar to pileipellis, a cutis with very rare to abundant erect hyphae, in places dense erect hyphae forming a trichoderm, repent hyphae hyaline, thin-walled, cylindrical, 1.5–12 µm broad, sometimes with lateral projections, erect hyphae (sub)cylindrical to filiform, hyaline, thin-walled, up to 150 µm long, 2–3.5 µm broad. Refractive hyphae scattered in trama of whole basidioma. Tramal hyphae non-amyloid and non-dextrinoid. Clamp connections absent.

Habitat: Lowland forest of *Alnus glutinosa, Fraxinus angustifolia, Acer campestre, Corylus avellana, Staphylea pinnata, Sambucus nigra,* and *Salix* sp., on soil.

Collections examined: CROATIA, vicinity of Novo Čiče village (near the town of Velika Gorica), 45°42'42"N, 16°06'54"E, alt. 103 m, leg. A. Mešić, Z. Tkalčec & M. Čerkez: 8 July 2002 (CNF 1/2633), 13 July 2002 (CNF 1/2639, 1/2643), 20 August 2002 (CNF 1/2677), 8 July 2009 (CNF 1/5565, 1/5566).

Additional collection examined: USA, Michigan, Oakland County, Kent Lake, leg. A. H. Smith, 24 September 1940 (MICH 15462, holotype).

Discussion

Gerhardtia piperata is characterized by its tricholomatoid habit, large (≤120 mm broad) pileus, predominantly cream colored basidiomata, adnate to decurrent lamellae, almost immediately acrid taste, subcylindrical to subfusiform spores with undate to nodose surfaces, siderophilous basidia, and absence of clamp connections. The most similar species is *G. suburens,* which differs mainly by a smaller (≤50 mm broad) pileus, taste that becomes sharp after ca. 30 seconds, distinct smell like *Lycoperdon* spp., and pileipellis that is a cutis lacking erect hyphae. Other species of the genus can be differentiated easily by a non-acrid taste.

According to our knowledge, *Gerhardtia piperata* hitherto has been found at seven localities in four countries: USA (Smith 1944), Germany (Bon 1979), France (Hertzog 1999, 2000; Wilhelm 2009), and Croatia. In USA, it was recorded five times at three different localities (Pontiac, Kent Lake, Dexter) in Michigan between 1937 and 1942 (from 31 July to 24 September) around or on old stumps and logs in low hardwood forests. The species was later found on 5 September 1975 northwest of Freiburg in southwestern Germany in an *Alnus-Fraxinus-Ulmus* flood forest. In France, *G. piperata* was found twice in Alsace province: near Ohnenheim on 24 September 1998 and in September 1999 in an

Alnus-Fraxinus-Populus-Ulmus flood forest and near Colmar on 12 September 2008 in a *Quercus-Carpinus betulus* dry forest. The distance between German and French localities is less than 30 km. In Croatia, it has been recorded eight times in 2002 and 2009 (from 8 July to 7 September) at one locality near Velika Gorica in the northwest part of the country in a lowland forest containing *Alnus glutinosa, Fraxinus angustifolia, Acer campestre, Corylus avellana, Staphylea pinnata, Sambucus nigra,* and *Salix* sp.

Although our observations correspond fairly well with the descriptions of *Gerhardtia piperata* in the literature, there are some differences:

(i) The Smith (1944) protologue gives basidiospore dimensions as 4–5.5 (–6) × 2–2.5 µm, which is not in accordance with either other descriptions or observations from the holotype made by Bigelow (1965, 1985). Our spore measurements from the holotype agree exceptionally well with those from the Croatian material: (5.3–)5.3–*6.4*–7.4(–8.6) × (2.1–)2.1–*2.4*–2.7(–2.8) µm, SD = 0.53 × 0.16, Q = 2.16–*2.65*–3.19.

(ii) Smith's statement that clamp connections are rare contradicts all other observations of the species. Neither Bigelow nor our studies of the holotype confirmed the existence of clamp connections.

(iii) Bon (1979), Contu & Consiglio (2005), and Hertzog (1999) described the spore print color as white; the color of two spore prints from Croatian material is yellowish cream.

(iv) We note the existence of metachromatic spores in Croatian material and in the holotype that is not mentioned by other authors.

(v) Basidiomata of *G. piperata* have similar cylindrical to filiform erect hyphae on the surface of the pileus and stipe, as well as on the lamellar edge, which are of different length and density. Smith (1944) used the term "projecting hairs" for erect hyphae on the pileus while Bigelow (1965, 1985) and Contu & Consiglio (2005) referred to them as "pilocystidia" and "pileocystidia", respectively. Bon (1979) and Hertzog (1999) described the pileipellis as a cutis with transitions to a trichoderm, and we share their opinion. Bon (1979), Contu & Consiglio (2005), and Smith (1944) stated that cheilocystidia were not present, while Bigelow (1965, 1985) and Hertzog (1999) did not mention cheilocystidia at all. Only Bon (1979) described erect hyphae on the lamellar edge projecting from the subhymenium or hymenophoral trama and called them marginal hyphae. For these elements we use the term cheilocystidia.

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